Final Phase II Sampling Design for
Fish and Invertebrates in Calcasieu Estuary

1.0 Introduction

The purpose of this document is to develop a sampling design for Phase II of the Remedial Investigation (RI) portion of a Remedial Investigation/Feasibility Study (RI/FS) being conducted for the Calcasieu Estuary in the Calcasieu Parish, Louisiana Estuary. The study area includes four general areas of concern (AOCs): the Bayou d'Inde, the Bayou Verdine, the Upper Calcasieu, and the Middle Calcasieu (Figure 1). Fish and invertebrate sampling and analysis in the Bayou Verdine AOC has been conducted by Conoco, and thus will not be considered further in this document. The Lower Calcasieu River is less contaminated than the four AOCs and can be considered a reference area. Another reference area, the Sabine National Wildlife Refuge (NWR), has also been identified for this RI. The Sabine NWR is located to the west of the Calcasieu estuary near the Texas border. It has relatively little industry, although it is connected to the Calcasieu estuary via an intracoastal waterway (Figure 2).

The Bayou d'Inde AOC includes three sub-areas: the lower (closest to the estuary), middle, and upper Bayou d'Inde. Heavy industry dominates the lower and, to a lesser extent, middle Bayou d'Inde (Figure 3). The Upper Calcasieu AOC includes the Lake Charles, Clooney Island, Clooney West Slip, Coon Island Northeast, and Coon Island Southwest sub-areas (Figure 4). Heavy industry also dominates these sub-areas, with the exception of Lake Charles, which is surrounded by the city of Lake Charles. The Middle Calcasieu AOC includes Prien Lake and the Old River Channel, Moss Lake, Bayou Olsen and the Citgo Surge Pond (Figure 5). The Lower Calcasieu River reference area includes Choupique Bayou and wetlands, Bayou Connine Bois, and Grand Bayou and wetlands (Figure 6), and is much less industrialized than the Upper and Middle Calcasieu AOCs.

A Phase I sampling program of fish and invertebrate tissues was conducted in early 2000. This sampling program included a number of fish and invertebrate species, although large, predatory fish species dominated the dataset (e.g., black drum). Samples were taken from a number of sub-areas within the Bayou d'Inde, Upper Calcasieu and Middle Calcasieu AOCs, and the Sabine NWR. Each sample was analyzed for a broad suite of metals and organic chemicals, and a subset of samples was analyzed for dioxin and furan congeners. Unfortunately, because the detection limits for the organic chemicals were too high, the Phase I database was dominated by non-detects, limiting the utility of the dataset for identifying chemicals of potential concern (COPCs) or using the results to estimate wildlife exposures. For some of the organic chemicals (e.g., the Aroclors), the detection limits were orders of magnitude above levels associated with effects to wildlife (Moore *et al.* 1999). As a result, the Phase II sampling program will serve two purposes: (1) the data will be used in a screening level ecological risk assessment (SLERA) to identify which chemicals and locations potentially pose risks to the wildlife assessment endpoints identified at our recent workshop (MacDonald et al. 2000), and (2) the data on chemical levels in fish and invertebrate tissues will be used as inputs to wildlife exposure models in the baseline ecological risk assessment (BERA). Our primary focus in this document is to identify which fish and invertebrate species should be sampled, where the samples should be taken, and to estimate the number of samples required for each species and location.

At a recent workshop held to support the baseline ecological risk assessment for the RI/FS of the Calcasieu estuary (MacDonald et al. 2000), the following ecosystem objectives were established that have direct relevance to the design of the Phase II sampling program:

- Maintain and, if necessary, restore aquatic environmental conditions that will support a healthy and diverse fish community;

- Maintain and, if necessary, restore aquatic, wetland, and terrestrial habitats that will support healthy, diverse, and self-sustaining populations of aquatic dependent avian species; and

- Maintain and, if necessary, restore aquatic, wetland, and terrestrial habitats that will support healthy, diverse, and self-sustaining populations of aquatic dependent mammalian species.

These ecosystem objectives were then used as the basis for selecting assessment endpoints and potential focal species that are representative of the assessment endpoints in the Calcasieu estuary:

- Survival, growth and reproduction of carnivorous fish:
  - black drum
  - red drum
  - sea trout
  - largemouth bass

- Survival and reproduction of insectivorous/sediment-probing birds:
  - willet
  - sandpipers
  - stilts
  - lesser scaup

- Survival and reproduction of carnivorous wading birds:

- great blue heron
- great egret
- spoonbills
- ibis

• Survival and reproduction of other piscivorous birds:
  - belted kingfisher
  - osprey
  - brown pelicans
  - terns

• Survival and reproduction of piscivorous mammals:
  - dolphins
  - mink
  - river otters
  - raccoons

Developing an appropriate Phase II fish and invertebrate sampling design for the Calcasieu estuary is a complex task. The fish and wildlife species that are the focus of this assessment range from relatively small predators with limited foraging ranges (e.g., kingfishers) to large predators with broad foraging ranges (e.g., black drum, dolphins). The preferred diets of the fish and wildlife focal species range from invertebrates (e.g., sandpipers) to small fish (e.g., kingfishers) and large fish (e.g., river otters, dolphins). Several wildlife focal species are highly opportunistic feeders (e.g., raccoons, mink). Potential prey species are highly variable in terms of their preferred habitat (e.g., sheltered bayous versus open water), size, mobility, and foraging range. The point estimates or distributions that will be developed in the BERA for prey tissue concentrations in the wildlife exposure models need to be appropriately scaled. For example, kingfishers have small foraging ranges and feed on small fish, which themselves have small ranges. Intuitively, we would expect the prey of the kingfisher to exhibit high spatial variability in tissue residues depending on proximity to sources. Because neither the kingfisher nor its prey spatially average their exposures, separate exposure analyses will be required for kingfishers inhabiting different sub areas in the Calcasieu estuary. Conversely, for wildlife species that forage widely (e.g., dolphins) on prey that themselves spatially average their exposures (e.g., black drum), we would expect that a single exposure analysis for the estuary would suffice. These two examples require quite different Phase II sampling designs. For kingfishers, we would want to sample small fish from a number of locations using a "randomized block" design (i.e., x randomly chosen samples from each of y sub areas – the size of the sub area scaled approximately to the foraging range of kingfishers). For dolphins, we could randomly sample large fish from the entire estuary. Given the broad array of wildlife focal species and prey being considered in this assessment, designing a Phase II sampling program will not be easy.

In this document, we will use two approaches to develop a Phase II sampling design. First, we will consider the feeding ecology of the fish and wildlife focal species and attempt to determine likely prey species (or types), and their likely foraging areas. The objective of this section is to determine what sizes and types of fish and invertebrates need to be collected, and to determine whether these collections should focus on specific local areas (the case for a predator with a small foraging range that feeds on sedentary prey species) or can simply be randomized over a larger portion of the estuary (the case for a broadly foraging predator that feeds on highly mobile prey species). Second, we will conduct statistical analyses on the tissue residues data collected during the Phase I sampling program. The objective of these analyses is to determine numbers of samples that need to be collected in Phase II to reliably develop measures of centrality and variability over the spatial scales of interest for the different predator-prey combinations. The statistical analyses, however, are somewhat limited because the Phase I dataset is dominated by non-detects as a result of the above-mentioned problem of high detection limits for many of the organic chemicals (e.g., Aroclors, dioxins, and furans). Also, the Phase I data set has insufficient sample sizes for invertebrates and small fish species to permit useful statistical analyses. The final section of this document combines these two approaches and proposes a recommended design for the Phase II sampling program in the Calcasieu estuary.

2.0 Feeding Ecology of Fish and Wildlife Focal Species and Their Prey

In the following sections, we review the feeding ecology of fish and wildlife focal species and their prey (also see Tables 1 and 2). The purpose of reviewing the feeding ecology of the fish and wildlife focal species is to better understand their diets, foraging ranges, and preferred foraging areas. This information is critical in designing the Phase II sampling strategy because, we would expect that different prey items will have quite different levels of chemicals depending on trophic level (particularly for bioaccumulative chemicals), proximity to sources, and foraging habits. Thus, samples of one prey item cannot be automatically substituted for others. The purpose of reviewing the ecology of prey species is to obtain a better understanding of their sizes, trophic level, and preferred habitat so that we can match the prey species to the appropriate fish and wildlife focal species. We will also categorize prey species into groups that share similar size ranges, habitats, and trophic levels. This categorization will facilitate easy substitution of prey species in the field, should particular species not be available.

2.1 Wildlife Focal Species

2.1.1 Insectivorous/Sediment-probing Birds

Willet (*Cataptrophorus semipalmatus*)

The willet is a pigeon-sized (*i.e.*, approximately 38 cm) member of the sandpiper family that weighs between 200 and 300 gm. The sexes are similar, however, the female is typically a bit larger. It breeds in southwestern Canada, the United States, and the West Indies (Knopf 1977). Willets are relatively rare in the Calcasieu estuary.

The willet's preferred habitat includes coastal marshes, sand dune areas, mudflats, and rocky zones. When nesting, willets often form small, loose, breeding colonies (Hayman et al. 1986). Their nesting and feeding territories are separate. The female chooses the nesting site, which is often concealed by short, thick vegetation, on a high, dry grassy area along a salt marsh, or on an open beach or flat. The nest itself is usually lined with dry grasses, a few dead rushes, or other materials nearby (Ehrlich et al. 1988). Feeding sites for willets are on sandbars, mud flats, and along tidal creeks and pannes of salt marshes.

Willets feed on small fish, aquatic insects, marine worms, small crustaceans and molluscs (Ehrlich et al. 1988). Similar to the sandpiper, the willet pecks items from the surface, and probes the mud with its bill, or stalks larger game in the water to catch their prey, sometimes capturing small fiddler crabs (Hayman et al.1986). Northern breeders are migratory, with the eastern birds moving southwards along the Atlantic seaboard and into the Carribean. In the winter, willets are found on or near the ocean shore (Hayman et al. 1986). The life expectancy of a willet is eight to ten years.

Spotted Sandpiper (*Actitis macularia*)

The spotted sandpiper is a small shorebird (approximately 19 cm), commonly found in Northern Alaska and Canada to the southern United States (EPA 1993; Knopf 1977). The average weight of a female sandpiper is 50 gm, which is significantly heavier than their male counterparts with an average weight of 40 gm (EPA 1993).

Spotted sandpipers preferred habitat is almost any place near water, such as lakes, rivers, reservoirs, and mountain streams, in both open habitats and wooded areas (Knopf 1977). Their nests are usually well concealed on the ground and lined with vegetation and hidden by grasses, or among rocks and driftwood. Sandpipers will walk slowly along the shores of sandy beaches, and the muddy edges of inlets and creeks, picking up food along the way (EPA 1993). Inland, the sandpiper feeds along the shores of sandy ponds, streams, and mountain torrents, at times straying into meadows, fields or gardens in agricultural areas, where they find their food in low vegetation or off the ground (EPA 1993; Bent 1962).

Spotted sandpipers generally feed on prey swimming close to the surface of the water (i.e., in the first 4 cm of the water column). They feed almost exclusively on small invertebrates, small fish, and both terrestrial and aquatic insects (e.g., beetles, grasshoppers, and ants). The sandpiper has the ability to capture flying insects, however, it prefers to catch its prey by probing and gleaning it from the substrate (EPA 1993; Bent 1962). The spotted sandpiper winters along the coasts in southern United States to South America, and breeds across North America, north from Virginia and southern California (Knopf 1977; EPA 1993). Spotted sandpipers are relatively common in the Calcasieu estuary during the spring and fall migration periods, but are not common otherwise.

Black-necked Stilt (*Himantopus mexicanius*)

The black-necked stilt is a medium sized shorebird (i.e., length 33 - 40 cm) found throughout southern and western United States, and south to Peru.

The preferred habitat of stilts is along coastal salt marshes, commercial saltpans, inland saltwater or freshwater lakes, mud flats, grassy marshes, or sewage farms (Hayman et al. 1986). Typically, black-necked stilts nest in small, loose colonies, in a shallow depression lined with grass, near a marsh or other wetland habitat (Knopf 1977).

Stilts are visual feeders, picking most of their food from the surface and shallow waters (i.e., in the top 20 cm of the water column). Their diet includes brine flies, brine shrimp, crayfish, snails, tadpoles, and some seeds, with the largest part of their diet coming from aquatic invertebrates, and the remainder from fish, reptiles and amphibians (Ehrlich et al. 1988). In the winter, they move to coastal zones, south of the United States (Knopf 1977).

Lesser Scaup (*Aythya affinis*)

The lesser scaup is a small to medium sized (i.e., length 40-42 cm) compact diving duck. It breeds in Alaska, western and central Canada, and the northcentral and northwestern United States, and winters throughout the southern and eastern United States, and Mexico. The lesser scaup is much less common along the coasts than the greater scaup.

The lesser scaup is often found on lakes and ponds in water no deeper than three metres. Nests are built by the female during egg-laying using dry vegetation and lined with grass, placed in a dry, upland location within 45 cm of the water, and usually very well hidden.

The lesser scaup is an excellent diver and a rapid underwater swimmer. They make shallow foraging dives and tend to feed in open water 3 - 8 m deep. The lesser scaup feeds on a mixture of plant and animal matter from the bottom substrate and the water column, including widgeon grass, seed from pondweeds, aquatic invertebrates, crustaceans and aquatic insects. Ducklings eat the same food as their parents, and are able to dive from a young age.

2.1.2 Carnivorous Wading Birds

Great Blue Heron (*Ardea herodias*)

The great blue heron is the most widely distributed and largest heron found in North America. It weighs approximately 2.9 kg when full grown. The heron is found in both freshwater and marine environments with their preferred habitat being freshwater lakes, rivers, brackish marshes, lagoons, mangroves and coastal wetlands. The size of the foraging territory depends on season. For example, EPA (1993) reports on a study where adult herons had a feeding territory of 0.6 ha in fall and 8.4 ha in winter. Heron nests are generally found close to foraging areas and tall trees are the preferred nest support (EPA 1993). Herons have been observed to defend their home territories, but have also been known as opportunistic foragers with little fidelity to foraging sites (EPA 1993). Herons are migratory birds and move to the southern Atlantic and Gulf states to overwinter.

The diet of the great blue heron consists primarily of small fish but they are known to consume amphibians, reptiles, crustaceans, insects, birds, and mammals (EPA 1993). The primary hunting strategy of the heron is to stand in shallow water and wait for fish to swim within striking distance. They usually fish is shallow waters (up to 0.5 m) with a firm substrate. Prey fish size is usually less than 25 cm in length (EPA 1993). Numerous field studies on great blue herons have recorded the size of prey consumed. Alexander (1977) found that most fish consumed were 8 to 33 cm long for 38 herons monitored in Michigan. Kirkpatrick (1940) found that the size of whole fish or fragments ranged from 6 to 41 cm, with the majority between 6 and 23 cm. In a survey of field studies examining trophic level of Great blue heron prey, EPA (1995) found that approximately 90% of prey were from the aquatic environment with trophic levels ranging between 2.4 and 3.0. The remaining prey (10%) were from wetland or terrestrial sources with trophic levels ranging from 1 to 3.5.

Great Egret (*Casmerodius albus*)

The great egret is a member of the heron family. It is approximately 89 - 104 cm in length, weighing approximately 1 kg, and occurs in southern Canada from Maine west to the Great Lakes, south to Texas, the Gulf Coast states, Florida and along the Atlantic coast (Knopf 1977).

The preferred habitat of the great egret is along freshwater and saltwater marshes, ponds, streams, lakes, bays, wooded swamps, mud flats, and urban environments (Ehrlich et al. 1988). Great egrets typically nest in colonies with other heron species in wetlands and wooded swamps. Nests are typically a large, flat platform constructed of sticks and twigs built in medium-sized trees, 7 to 13 m above ground, or sometimes built in bushes that are less than 2 m above water, usually found near a foraging site (Ehrlich et al.1988).

Great egrets feed primarily on fish and invertebrates. Part of their diet may include crayfish, frogs, other aquatic organisms, small birds, crickets, grasshoppers, snakes, and mice (Erwin 1984). Their prey size varies, but seldom exceeds 1 m in length. The nestlings are usually fed frogs, crayfish, and small fish that are regurgitated into their mouths.

Great egrets forage alone or in groups by walking slowly in shallow water. Sometimes they forage with other species, occasionally stealing from the smaller species (Ehrlich et al. 1988). Great egrets spend the winter from South Carolina southward, and in the summer can be found as far north as Massachusetts. Some banded birds have lived more than twenty-two years.

Roseate Spoonbill (*Ajaia ajaja*)

The spoonbill is a large bird (i.e., 76 - 81 cm), found on the coasts of southern Florida, Louisiana, and Texas, as well as Mexico, West Indies, and Central and South America (Knopf 1977).

Its preferred habitat is along coastal marshes, lagoons, estuaries, and mudflats. They typically nest in colonies with other species (e.g., ibises, herons, and egrets). They build large nests in trees constructed with branches and plant stems, lined with leaves, grasses, and bark.

Spoonbills have a unique foraging strategy. They step through the water up to 20 cm deep swinging their bill from side to side with their jaw slightly ajar until the highly sensitive nerve endings inside their bill detect prey. When their prey is sensed, the two paddles snap shut and, with a toss of the head, the prey is consumed. They feed on shrimp, molluscs, fish, sheepshead minnows, and insects. Nestlings fledge at about six weeks of age. Spoonbills winter in southwestern United States and Gulf coastlines. The roseate spoonbill has a life expectancy of ten years in captivity.

Ibis

The white ibis (*Eudocimus albus*), glossy ibis (*Plegadis falcinellus*), and white-faced ibis (*Plegadis chihi*) are medium to large sized birds (i.e., 56 - 68 cm), found along the coastal islands from South Carolina to Florida, Louisiana, and Texas (Knopf 1977).

Preferred habitats for the ibis include marshes, swamp forests, bays, ponds, lagoons, mud flats, coastal bays, flooded fields, and salt marshes (Knopf 1977). They generally nest in colonies, often mixed with egrets and herons, typically in trees or shrubs over the water, or on the ground in a low bush or marsh area. Nests are always guarded and usually constructed with sticks, aquatic vegetation, twigs, roots, and leaves, lined with finer materials (Knopf 1977; Ehrlich et al. 1988).

Food preferences include aquatic invertebrates, crayfish, crabs, fish, fiddler crabs, insects, earthworms, water snakes, and snails (Ehrlich et al. 1988). Foraging is often accomplished commensally with snowy egrets or other bird species.

2.1.3 Other Piscivorous Birds

Belted kingfisher (*Ceryle alcyon*)

The belted kingfisher is a medium sized bird (average length = 33 cm), commonly found in most of North America (NGS 1983). Belted kingfishers are primarily winter residents in the Calcasieu estuary. Kingfishers prefer habitat along stream, lake or pond edges as well as sea coasts and estuaries (Prose 1985). They require water that is clear and not obscured by overhanging tree canopies or aquatic vegetation. Belted kingfishers nest in burrows in earthen banks beside rivers, streams, ponds and lakes. They have also been found in slopes created by human excavations such as roadcuts and landfills (FWS 1985). Kingfishers nest near suitable fishing areas when possible, but will nest away from water and feed in bodies of water other than the one closest to home. During spring and early summer, both male and female kingfishers defend a territory that includes both their nest site and their foraging area (EPA 1993; Prose 1985). By autumn, each bird defends an individual feeding territory only. Breeding territories are on average more than twice as long as non-breeding territories (i.e., 1,030 ± 219 m vs. 389 ± 92.6 m, respectively) (Prose 1985).

The belted kingfisher diet consists primarily of fish and occasionally invertebrates (e.g., crayfish, insects) (EPA 1993). During shortages of their preferred foods, they have been known to consume crabs, lizards, frogs, turtles, mussels, small snakes, insects, salamanders, newts, young birds, mice, and berries (Prose 1985). Belted kingfishers generally feed on fish that swim close to the surface or in shallow water (i.e., in the first 12-15 cm of the water column) (EPA 1993). Feeding strategies vary depending upon the availability of perches over the water body. A typical approach is to capture fish by diving from a perch overhanging the water. Kingfishers may also hover over the water and wait for potential prey to come into view (EPA 1993; Prose 1985). Several field studies have reported on the size preference of fish caught by belted kingfishers. Salyer and Lagler (1946) reported the average length of fish caught in a field study in Michigan was less than 7.6 cm (range: 2.5 - 17.8 cm). Davis (1982) found that belted kingfishers in Ohio selected fish ranging from 4 - 14 cm and 88% of the fish were between 6 - 12 cm. The trophic level of the prey consumed by belted kingfishers varies slightly between regions. In a survey of field studies examining trophic level of kingfisher prey, EPA (1995) found that 94% of prey were from the aquatic environment with trophic levels ranging between 2.6 and 3.

Osprey (*Pandion haliaetus*)

Osprey are large birds of prey found worldwide. The mean body weight of a female Osprey is 1.6 kg while the male is slightly smaller at 1.4 kg (EPA 1993). Body length ranges from 53 - 61 cm (EPA 1993). The osprey population experienced a marked population decline in the 1960s and 1970s due to exposure to persistent, bioaccumulative chemicals (e.g., DDT). Conservation programs and the reduction and/or elimination of DDT has halted this decline. Ospreys are now fairly common in coastal areas (NGS 1983).

Osprey prefer marine environments or large inland lakes, rivers, and estuaries. Nesting sites are typically found close to open, shallow waters with a large abundance of prey (EPA 1993). Isolated trees and man-made structures are preferred nesting sites for osprey. Ospreys will travel up to 15 km to obtain food (EPA 1993). The typical foraging radius for osprey is 1.7 - 10 km (EPA 1993).

Osprey are piscivorous birds and prey preferences change seasonally with abundance of local fish (EPA 1993). Osprey occasionally prey on birds, frogs, and crustaceans (EPA 1993). Osprey feed twice a day, in the mid-morning hours and again in late afternoon and they consume roughly 300 g of food per meal (Watkins 2000). They are most successful at catching slow moving fish in shallow waters, and fish that remain close to the surface (EPA 1993). All parts of the fish are consumed with bones and indigestible parts being ejected in faecal pellets (EPA 1993). Osprey feed on medium sized fish (i.e., 11 - 30 cm) (USGS 2000). Collopy (1984) observed the prey items caught by osprey from four nests in Florida from March to June. They found gizzard shad, sunfish, largemouth bass and golden shiner were the primary prey items. Gizzard shad and sunfish ranged from 15 - 20 cm and 12 - 16 cm in length, respectively. French (1972) observed a number of osprey found in California and found they were primarily feeding on surf smelt and night smelt that ranged from 13 - 15 cm in length. Van Daele and Van Daele (1982) examined the size of fish caught by osprey in an Idaho reservoir. They found that osprey caught fish <10 cm 3.3% of the time, 11 - 20 cm 42.1% of the time, 21 - 30 cm 46.7% of the time, and larger fish (i.e., 31 - 41+ cm) 7.9% of the time. In a survey of field studies examining trophic level of osprey prey, EPA (1995) found that approximately 95% of prey were from the aquatic environment with trophic levels ranging between 2.5 and 3.1.

Brown Pelican (*Pelecanus occidentalis*)

The brown pelican is a large, stocky bird (i.e. 114 - 137 cm), found on the Atlantic Coast from North Carolina south to Venezuela, and on the Pacific Coast from British Columbia to Chile (Knopf 1977). The eastern population of the brown pelican had experienced a decline which was primarily caused by the ingestion of pesticide residues (*e.g*. DDT) in their prey.

Brown pelicans prefer nesting sites on small coastal islands that provide protection from mammal predators (e.g., raccoons, coyotes), with sufficient elevation to prevent flooding of nests. They are known to fly, roost, nest, and fish in groups. Pelicans tend to nest either on the ground, in mangrove trees, or in bushes. Ground nests vary from practically nothing to well built retreats of sticks, reeds, straws, palmetto leaves, and grasses. Tree nests are made of similar materials.

The brown pelican is primarily a fish eater, and requires up to 2 kg of fish per day. Young pelicans are fed for approximately nine weeks and during this time each individual will consume roughly 70 kg of fish. Pelicans typically eat “rough” fish, species considered commercially unimportant, such as menhaden, herring, sheepshead, pigfish, mullet, grass minnows, and silversides. Feeding typically occurs in shallow estuarine waters with the birds seldom venturing more than 30 km out to sea except to take advantage of especially good fishing conditions. The brown pelican searches for its prey by flying slowly above the water surface at varying distances, and using their extremely keen eyesight to spot a single fish or a school of small fish. Once they spot their prey, they dive into the water, sometimes submerging completely. When their bill enters the water, the highly expandable gular pouch is filled with water and becomes enlarged, and when the bill is closed it may contain as much as 10 litres of water along with their prey. If the pelican is successful, it will lift its closed bill slowly which allows the water to flow out of the pouch, leaving the prey inside. With a tossing motion of the head the prey is then swallowed (Arnqvist 1992). Typically, a brown pelican can live up to thirty years or more.

Terns

Terns are generally small to medium sized birds found in close proximity to aquatic environments. There are numerous tern species found in North America. The Sandwich tern, Least tern, and Forster's tern winters from the Gulf Coast and Central America south to Peru, Brazil, Africa, Guatamala, and India (Knopf 1977). They migrate in small, loose groups taking rests on sandbars, beaches, docks and pilings to feed. Two tern species are likely to be found in the area surrounding the Calcasieu Estuary: the Forster's tern and Gull-winged tern.

The Forster's tern (*Sterna forsteri*) prefers open marine and freshwater habitats and is typically found along coasts. Less commonly, it may be found in wet prairies and agricultural environments. It is approximately 35 cm tall (NGS 1983). The preferred nesting sites for Forster's terns are fresh and saltwater marshes. The Gull-winged tern is generally slightly smaller than the Forster's tern and prefers to nest in salt marshes and on beaches (NGS 1983).

Terns tend to feed on small fish, such as sandlances or sand eels, and crustaceans, like shrimp and prawns. The Caspian tern will also eat larger fish such as mullet and menhaden. They capture their aquatic prey by skimming the surface of the water or by hovering over the water then diving head first into the water catching their food with their bills. The Gull-winged tern is often seen hunting for insects in fields and marshes (NGS 1983). Baltz et al. (1979) examined the stomach contents of 15 Forster's terns in California to determine their preferred prey. Approximately 95% of the stomach contents consisted of various small fish species including shiner perch, northern anchovy, night smelt, top smelt, arrow goby, and other unidentified gobies.

2.1.4 Piscivorous Mammals

Bottlenose Dolphin (*Tursiops truncatus*)

The bottlenose dolphin is a stout, short-beaked, long (i.e., ranges from 3 to 4 m long), highly intelligent aquatic mammal. It is found in coastal and offshore waters in temperate to tropical seas. There are two ecotypes of bottlenose dolphins found in the U.S. Atlantic coastal waters: the inshore/coastal type, and the offshore type. The inshore dolphin is the most abundant marine mammal along the Virginia and North Carolina coasts. Populations of these dolphins occur along the coast and inshore waters from northern Cape Hatteras to southern Florida and west through the Gulf of Mexico. The offshore population can be found as far north as New Jersey, living along the edge of the continental shelf. The bottlenose dolphin, which occurs from April through November in Virginia coastal and estuarine waters, is considered part of the coastal migratory stock of Atlantic bottlenose dolphins.

Dolphins live and socialize together in groups ranging from 10 to 100 inshore, and up to several hundred offshore. The preferred habitat of the inland bottlenose dolphin includes waters with a depth of less than 30 m. Habitats occupied by the bottlenose are diverse, ranging from calm, shallow lagoons to rocky reefs, and open waters. They are commonly seen in bays, estuaries, harbours, lagoons, river mouths, and ship channels.

Bottlenose dolphins are opportunistic feeders, eating a wide variety of fish and invertebrates, including squid, minnows, eels, menhaden, catfish, tarpon, sailfish, speckled trout, pike, rays, and mullet. They also forage on shoaling and bottom-dwelling species, which includes an assortment of fishes, squid, and octopus. They consume about 18 - 36 kg of fish each day, ranging in size up to 3.4 m.

Bottlenose dolphins typically forage and feed in groups, herding schools of fish. At times, they will forage on their own, chasing their prey individually onto mud banks. The average lifespan of the bottlenose dolphin is thirty-five years.

Mink (*Mustela vison*)

The mink is the most abundant and widespread carnivorous mammal in North America. The male is usually larger than the female, sometimes weighing twice as much as the female (EPA 1993). Mink are found in all parts of Canada, the United States and Alaska.

The mink is a nocturnal mammal found in a variety of wetland and riparian habitats. Its preferred habitat is along waterways such as rivers, lakes, streams, ditches, swamps, river banks, ponds, backwater areas, and fresh and saltwater marshes (EPA 1993; Allen 1984). Key factors for a good mink habitat include permanent water, adequate shoreline vegetation, and a constant food source. Mink are territorial individuals defending linear territories of 1 to 4 km of river or lakeshore by scent marking and overt aggression. Within this territory is a core area where the occupant forages most intensively. Mink prefer irregular shorelines to more open, exposed banks, and they also tend to use brushy or wooded cover adjacent to the water where cover for prey is abundant and where downfall and debris provide den sites (EPA 1993).

When foraging, they become aggressive predators and often attack animals larger than themselves. Mink rely on their sense of smell to find prey. Mink are not well adapted for swimming, and therefore concentrate search efforts for food in upland areas near the water, along shorelines, or in shallow water. Mink are opportunistic and will feed on muskrats, birds, fish, amphibians, crustaceans, and terrestrial prey such as mice and rabbits (EPA 1993). The average lifespan of a mink in the wild is less than three years.

Northern River Otter (*Lutra canadensis*)

The northern river otter is the largest member of the *Mustelidae* family that also includes mink, weasels, skunks, and badgers. River otters range from 5 to 10 kg in body weight and measure 66 to 76 cm in length with a 30 to 43 cm tail (EPA 1993). Male otters tend to be larger than females. The river otter is found throughout North America and in relative abundance in Alaska, most of Canada, the Pacific northwest, the Great Lakes region, and most states along the Atlantic and Gulf of Mexico coasts.

The otter prefers habitat close to lakes, marshes, streams, and seashores. Abundance of food is a primary consideration of the otter in selecting a habitat. The river otter dens in banks and hollow logs (EPA 1993). Home range size varies from 10 to 78 km of shoreline depending upon the stage of life of the otter and food/breeding site availability (EPA 1993).

The diet of the river otter primarily consists of fish, although they are known to be opportunistic and will feed on a variety of prey (e.g., aquatic insects, amphibians, insects, birds, mammals, and turtles) (EPA 1993). Otters may probe the bottoms of ponds or streams for invertebrates and thus ingest sediment and/or other debris in the process. EPA (1995) reviewed available field studies on river otter diet. Diet varied markedly depending upon the season in several of the field studies reviewed. For example, as fish may not be as readily available in some regions during the winter months (i.e., due to ice), river otters rely on other organisms in these areas. Because otters are fairly large mammals, and are opportunistic in their feeding habits, fish size can vary markedly. Melquist *et al.* (1981) reported that river otters consumed fish ranging from 7 to 45 cm in length. Fish species included mottled sculpin, largescale sucker, northern squawfish, yellow perch, mountain whitefish, and salmon. The trophic level of prey items consumed by the northern river otter varies depending on the source of the prey. Aquatic prey trophic levels typically range from 2.6 - 3.2, wetland prey trophic levels range from 2.0 - 4.0, and terrestrial prey range from 1.0 - 2.6 (EPA 1995).

Raccoon (*Procyon lotor*)

The raccoon is a medium-sized, widespread omnivore, commonly found in most of North America (EPA 1993). Raccoons are found in hardwood swamps, freshwater and saltwater marshes, mangroves, and floodplain forests. Raccoons are also common in residential areas and farmland, both cultivated and abandoned (EPA 1993). Preferred denning sites include hollow trees, logs, stumps, holes in the ground, caves, and rocky ledges. In rural areas, the home range of an adult male raccoon ranges between 1 to 4 km, whereas raccoons living in urban areas tend to utilize a home range of less than 0.1 km.

Raccoons are opportunistic feeders and will consume practically any food item whether plant or animal. Their diet includes small mammals, lizards, frogs, birds, squirrels, rabbits, crayfish, turtles, waterfowl eggs, insects, corn, fleshy fruits, nuts, berries, and grains (EPA 1993). A review of 19 field studies for raccoon diet was undertaken by EPA (1995). The range of prey trophic levels in the aquatic environment for all seasons ranged from 2.2 - 2.8. The range of wetland prey trophic levels for all seasons was 2 - 3.4. The range of terrestrial prey trophic levels for all seasons was 1 - 2.3. Average percent diet was determined based on the % volume of prey in the digestive tracts of the raccoons collected (EPA 1995). Terrestrial organisms dominated the diet of raccoons in most seasons (i.e., generally >70%), with aquatic and wetland organisms making up the difference. In spring, the diet was generally more evenly distributed between aquatic, wetland and terrestrial prey.

Raccoons are primarily active at night, travelling to where food is available (EPA 1993). They use their sensitive front feet to forage, catching their prey in and around the water or near crop fields. They hunt in shallow water by turning over rocks and limbs, probing and grabbing with their front feet.

2.2 Fish and Invertebrate Species

The fish and shellfish habitat of the southern Gulf of Mexico area includes marine, estuarine, as well as freshwater characteristics. The fish and shellfish species inhabiting these locations include not only purely marine or freshwater species, but also those that are tolerant to changes in salinity. The latter species are able to feed and sometimes reproduce in either habitat type. Estuarine species are also adapted to the shoreline habitat of the northern Gulf of Mexico, which typically includes shallow and overgrown backwaters with muddy or sandy substrate.

The following fish and shellfish families have geographic ranges that include the marine, freshwater, and estuarine habitats along shorelines, bayous, rivers, and lakes of Louisiana, Texas, and Florida (Table 2). The main source of the life history data is the fish database (FishBase) by R. Froese and D. Pauly. (FishBase. World Wide Web electronic publication.www.fishbase.org, 27 September 2000). Hoese and Moore (1998) was also consulted.

Family: Ariidae (Sea Catfishes)

Fish species in this family are mostly marine, but may enter freshwater. There are a few freshwater species. Their geographic distribution includes tropical and subtropical waters. Sea catfishes are typically found in turbid water over muddy bottoms, where they prey on zoobenthos.

Family: Atheridae (Silversides)

Silversides are mostly marine with distributions including tropical and temperate waters. There are about 50 species that are confined to freshwater and others that enter freshwater. Silversides feed on zooplankton in lakes, ponds, quiet pools of creeks, and along shores and bays. They typically swim near the surface, so are a potential target for terrestrial predators. The largest species can reach a maximum length of 60 cm.

Family: Blenniidae (Combtooth Blennies)

Blennies have a wide distribution including the Indian, Atlantic, and Pacific Oceans. They are a mainly tropical and a subtropical marine group of species. Blennies are somewhat rare in fresh and brackish waters. They are found near mangroves, pilings, rocky shores, boulders, and oyster reefs.

Blennies are typically bottom dwellers that feed on a mixed diet of algae and benthic invertebrates. Some are planktivores and others are specialized to feed on skin or fins of larger fish. Blennies grow to a maximum length of about 54 cm, however, most are smaller than 15 cm.

Family: Centrarchidae (Sunfishes)

Sunfish are typically found in North America. Sunfish are freshwater species that prefer quiet pools and backwaters of lakes, ponds, streams, and rivers. They usually occur in heavily vegetated areas with sand or mud bottoms and can tolerate turbid water. Sunfish are omnivores and feed on small fish, crayfish, frogs, crustaceans, and insects. They are preyed upon by herons, bitterns, kingfishers, and larger fish. Sunfish can grow to 83 cm maximum in length, but most are usually much smaller.

Family: Clupeidae (Herrings, Shads, Sardines, Menhadens)

This family has a global distribution, but prefers mostly tropical zones (from 70/N to about 60/S). This is largely a marine coastal group of species that tend to school. However, there are some species that live or enter freshwater and estuarine habitats. The fish feed in the water column or at the bottom where they consume small planktonic animals. Adult fish range in size from 2 to 75 cm.

Family: Cyprinodontidae (Pupfishes)

Pupfish are mainly found in freshwater and brackish environments of continental USA, Middle America, West Indies, parts of South America, northern Africa, and Mediterranean Anatolian region. There are some coastal marine species. Their preferred habitat type includes vegetated sloughs, ponds, lakes, and slow streams, often over muddy bottoms and in turbid water. Some are found on marine tidal flats in hypersaline lagoons and channels.

Pupfishes are omnivores with a diet consisting of worms, crustaceans, and plant matter. The predators include larger fish that grow to a maximum length of 22 cm.

Family: Engraulidae (Anchovies)

Schools of anchovies can be observed in shallow coastal waters and estuaries in tropical and temperate regions of the Atlantic, Indian, and the Pacific Oceans. Some species enter or live in freshwater. Anchovies are mostly filter-feeders and food items include mysids, copepods, small fish, gastropods, and isopods. The predators of anchovies include jellyfish and larger fish. Anchovies can grow up to 50 cm, but most species are below 15 cm.

Family: Fundulidae (Topminnows and Killifishes)

The distribution of topminnows and killifish includes the lowlands of North and Middle America, southeastern Canada, and Yucatan. They are found in the Mississippi River drainage basin as well as Bermuda, and Cuba. Many species are euryhaline. Their preferred habitat consists of shallow, sandy runs, pools and backwaters of creeks, rivers, and swamps. Topminnows and killifish like to swim several cms below the surface in heavily vegetated areas. Their diet is omnivorous.

Family: Gobiidae (Gobies)

This family contains the greatest number of marine fishes (possibly >2,000). Gobies are mostly marine and brackish. Some species are catadromous. Their geographic distribution includes largely tropical and subtropical areas. Their preferred habitat type includes very shallow coastal waters and tidal flats. Gobies often swim over muddy bottoms and in foul water. Gobies are omnivores and feed on benthic invertebrates and plankton. Some species have symbiotic relationships with invertebrates (e.g., shrimp) and others are known to remove fish ecto-parasites. The shallow-swimming fish are preyed upon by sea birds. Gobies grow to a maximum of 50 cm, but most species are below 10 cm.

Family: Lepisosteidae (Gars)

Gars are mainly freshwater species, but can enter brackish waters occasionally. They are rarely marine. Gars are found in eastern North America (from southern Quebec to Costa Rica) and Cuba. Their habitat consists of quiet, clear pools and backwaters of lowland creeks, rivers, lakes, swamps, and sloughs. They prefer shallow and weedy areas. The larger individuals can reach a maximum length of 3 m.

Family: Microdesmidae (Wormfishes)

Wormfish are found in tropical seas, but rarely in brackish or freshwater. They inhabit shallow waters of muddy estuaries and tidepools and often burrow in sand or mud. Wormfish hover above the substrate to feed on zooplankton. The fish can grow to a maximum length of 30 cm.

Family: Mugilidae (Mullets)

Mullets are a cosmopolitan group commonly found in coastal areas of the Atlantic, Indian, and Pacific Oceans. They are typically marine species, although a few species can enter brackish and freshwater habitats. Mullets like to feed over sandy or muddy bottoms of rivers, estuaries, bays, and inlets. Their omnivore diet consists of zooplankton, zoobenthos, detritus, and algae. Mullets are preyed upon by whales, dolphins, body fish, sharks, rays, and sea and shore birds. Mullets grow to a maximum length of about 90 cm.

Family: Paralichthyidae, Bothidae, and Pleuronectidae (Flounders), Achiridae (Soles), and Cynoglossidae (Tonguefishes)

Flounders, soles and tonguefishes are generally marine species, but some species can tolerate brackish or even freshwater. Flounders are common in estuaries, bays, lagoons, and shallow coastal waters. These families prefers sandy or muddy bottoms rather than hard substrate. Their omnivorous diet is composed of fish, shrimp, and invertebrates. They are preyed upon by larger fish.

Family: Poeciliidae (Poecilids)

Poecilids are found at low altitudes from eastern United States to northeastern Argentina. Although mostly freshwater, poecilids are commonly found in brackish and marine environments of coastal areas. Their preferred habitat includes ponds, lakes, sloughs, and quiet vegetated backwaters and pools of small rivers. The fish are abundant in tidal ditches and brackish canals. Poecilids feed mainly on algae, but also prey on worms and crustaceans. The fish are usually less than 18 cm in length.

Crabs

Crabs are a very common group of invertebrates along the shores of all marine bodies of water. Their preferred habitat includes shallow bays or estuaries covered with eel grass. Crabs range in size from less than a few cms (e.g., fiddler crabs) to greater than 30 cms (e.g., blue crabs). Crabs are generally predatory species.

Family: Sciaenidae (Drums and Croakers)

Drums and croakers are bottom-dwelling carnivores that feed on benthic invertebrates and small fishes. They have a wide geographic distribution that spans the Atlantic, Indian, and Pacific Oceans. They prefer coastal waters, especially large river runoffs. They also enter estuaries and brackish swamps. Drums and croakers are typically found over sandy or muddy bottoms. Their main food items are crustaceans and fish. Drums and croakers are preyed upon by larger fish.

3.0 Recommended Prey Sampling Strategy

The selected prey species can be organized into four logical groups each reflecting a particular combination of size range, distribution in the water column, trophic level, and foraging range. Below, we discuss each of these sorting criteria and the resulting prey groups. The grouped prey species are shown in Table 3.

Body Size

Fish and invertebrate species found in the southern USA vary widely in terms of body size. They range from tiny gobies (Gobiidae) less than a centimeter in length to alligator gars and drums that are up to 2 to 3 meters in length.

Water Column Distribution

The estuarine habitat os Southern Louisiana favors demersal fish. Tidal, coastal, swampy or marshy areas tend to be shallow and have mostly muddy bottoms that provide a good substrate and source of food for demersal fish prey such as crustaceans, annelids, molluscs, and algae. Thus, it is not surprising that the majority of fish species listed for this geographic location are bottom fish. Examples include catfishes (Ariidae), blennies (Bleniidae), Suckers (Catostomidae), some killifish (Fundulidae), gobies (Gobiidae), wormfishes (Microdesmidae), flounders (all families), and Rockfishes (Sebastidae).

Pelagic fish include silversides (Atherinidae), sunfishes (Centrarchidae), herrings (Clupeidae), pupfishes (Cyprinodontidae), anchovies (Engraulidae), killifishes (Fundulidae), gars (Lepisosteidae), mullets (Muglidae), poecilids (Poecilidae), and drums (Sciaenidae).

Trophic Level

Prey species at different trophic levels are likely to have substantially different levels of bioaccumulative chemicals in their tissues. There are prey species that are exclusively phytoplanktivores such as herring, shad, sardine, and menhaden (Clupeidae). There are also purely carnivorous species such as blue crab, bass (Centrarchidae), anchovy (Engraulidae), gar (Lepisosteidae), and most sebastids (Sebastidae). Most of the reported species, however, are omnivores.

The trophic level for planktivores is low at 2.0. For omnivores, trophic level ranges from 2.1 for mullets (Muglidae) to 3.4 for silversides (Atherinidae) and pupfishes (Cyprinodontidae). Carnivores may have even higher trophic levels.

Foraging Range

The foraging range of each prey species generally depends on foraging strategy, diet, and body size. A general observation is that larger fish tend to forage more broadly than do smaller fish. The latter group tends to find suitable habitat in quiet bays and inlets and remain there, whereas larger fish are more likely to enter open water and travel extensively in search for food. There are exceptions to this observation. Anchovies, for example, tend to be small, yet they cover large distances. Conversely, gars can be very large and yet tend to move little. Species with large foraging ranges are likely to exhibit less spatial variation in tissue residues than will species with small foraging ranges.

Prey Groups

Based on our review, we believe that the following five groups represent the range of prey species likely to occur in the Calcasieu estuary. For the Phase II sampling program, we believe that species within a particular group are (more or less) interchangeable. That is, predators will be unlikely to have distinct preferences for one species over another within a group. Further, we would expect that tissue residue levels will be similar between species within a group, providing they are collected in the same area.

Group 1. Small bentho-pelagic fish that inhabit shallow water and have a localized distribution. The trophic level ranges from 2 to 2.5. Tissue residue levels are expected to vary substantially between individuals depending on proximity to sources.

Group 2. Small pelagic fish that inhabit water of any depth and have a broad distribution. The trophic level ranges from 2 to 2.5. Tissue residue levels among individuals are not expected to vary widely between sub areas in the Calcasieu estuary.

Group 3. Large pelagic fish that inhabit water of any depth and have a broad foraging range. The trophic level ranges from 2 to 2.5. Tissue residue levels among individuals are not expected to vary widely between sub areas in the Calcasieu estuary.

Group 4. Medium to large bentho-pelagic fish that inhabit water of any depth and have a broad foraging range. The trophic level ranges from 2.5 to 3.5. Tissue residue levels are expected to be high for bioaccumulative chemicals, but are unlikely to vary widely between sub areas in the Calcasieu estuary.

Group 5. Large pelagic fish that inhabit shallow water and have a local distribution. The trophic level ranges from 2.5 to 3.5. Tissue residue levels are expected to be high for bioaccumulative chemicals in contaminated sub areas in the Calcasieu estuary. Otherwise, levels will be lower.

Each of the fish and wildlife focal species tend to feed upon one, or at most, two of the above prey groups. The appropriate Phase II sampling strategy varies depending on which prey group is of interest. For example, less samples are required for groups 2, 3 and 4 because, with their broad foraging ranges, we would expect spatial variation between individuals/species to be fairly limited (i.e., the individuals are spatially averaging exposures). More samples will be required for group 1 prey.

The majority of the fish and wildlife focal species feed upon prey in group 1, although several species also feed upon prey in group 2, 3, and 4 (Table 4). Focal species are unlikely to feed on prey species in group 5.

The analysis of feeding strategies of the focal species, leads us to the following recommendations for the Phase II sampling program:

- Sampling of small fish and invertebrates that tend not to move around much (i.e., group 1) is a crucial component of this sampling program if we are to develop credible exposure estimates for most of the fish and wildlife focal species. We believe that group 1 species will exhibit considerable spatial variation across the Calcasieu estuary and thus the sampling program will need to concentrate on obtaining sufficient samples from each of a number of sub-areas. The results from this kind of detailed sampling will permit us to develop separate exposure estimates in different sub areas for individuals of focal species with small foraging ranges (e.g., kingfishers).

- Sampling of fish species that move around a lot (i.e., groups 2 - 4) is also required for some wildlife focal species (e.g., osprey, dolphin). We believe that these species will exhibit less spatial variation across the Calcasieu estuary (see next section) and thus the sampling program will not require detailed sampling at the sub area spatial scale. For these groups, it should be sufficient to obtain sufficient samples at a higher level of resolution (e.g., upper Calcasieu, middle Calcasieu, lower Calcasieu, Bayou d'Inde).

- No sampling of group 5 fish species (i.e., gars) is required for the ecological risk assessment (although this may be a requirement for the human health risk assessment).

The next section explores the number of samples required for each of groups 1-4.

4.0 Sample Size

We examined the data from the Phase I sampling program to obtain a better understanding of variability within and between sub-areas, and to determine appropriate sample sizes for each prey group. Because of the problem with non-detects in the dataset, we were only able to consider several chemicals in this analysis (i.e., dieldrin, chlordane, copper, mercury and zinc). Also, the only species adequately represented in the dataset were group 3 and 4 prey species (i.e., black drum, sea trout, and, to a lesser extent, flounder and mullet). We expect that the patterns of spatial variation will be quite different for smaller fish and invertebrate species that do not move around much (i.e., group 1).

Figure 7 presents box plots of log (10) chemical tissue residue concentrations for species with multiple observations. The box represents the inter-quartile range of the data, the solid dot inside the box is the median. The whiskers extend to the farthest data points within 1.5 inter-quartile range from the 25th and 75th quantiles. The figure is arranged by species (columns) and chemicals (rows). The data indicate for these species that forage widely that the spatial variability between the general areas is relatively small (upper Calcasieu, middle Calcasieu, Bayou d'Inde and Sabine NWR). Somewhat surprisingly, the levels of several chemicals (e.g., dieldrin, zinc, copper) are similar between the Sabine NWR (the "reference" site) and locations within the Calcasieu estuary. This suggests that the sources of these chemicals are broadly distributed rather than arising from within the Calcasieu.

Figure 8 shows the coefficients of variation for each species-chemical combination. With the exception of black drum, most of the species-chemical combinations calculations were based on only a few samples. For black drum, the coefficient of variation within each general area was fairly consistent, and generally low (i.e., 0.4 to 1). This information can be used as the basis for power analyses to determine appropriate sample sizes.

Figures 9 to 11 show power curves for mercury, copper, and dieldrin in black drum and flounder at selected locations. The statistical power of detecting a given difference (shown in the legend in log units) is plotted against sample size. The power estimation is based on a significance level of 0.05 and we assume that chemical concentration follows a log-normal distribution. These curves are usually used as the basis for determining sample size. For example, a sample size of 50 is needed in order to test a log difference of 0.3 (i.e., factor of 2) for mercury tissue residues in black drum with a power of 0.80. Using the three selected species-chemical comparisons, a minimum of 20 samples is necessary to achieve a power of 0.8 with a modest log difference of 0.5 (i.e., factor of 3).

5.0 Recommended Sampling Design

Based on our review of the ecology of the focal species and their prey, we recommend that the sampling design for group 1 prey has a sub area spatial resolution. We also recommend that group 1 organisms be separate into two sub-groups, group 1a - small benthic invertebrates and group 1b - small benthopelagic fish. This refinement to the classification system is recommended to ensure that sufficient data are collected on aquatic invertebrates to adequately evaluate risks to those fish, bird, and mammal species that rely on group 1 invertebrates as a substantial portion of their diet. For groups 2, 3 and 4, a general area spatial resolution is adequate. Whether at the sub area or at the general area spatial resolution, a minimum of 20 samples is required if we are to eventually generate credible exposure estimates. Therefore, for each of groups 2, 3 and 4, we recommend that a minimum of 20 samples be taken in each of four general areas (upper Calcasieu, middle Calcasieu, lower Calcasieu, Bayou d'Inde). Thus, a minimum of 80 samples are required from each of groups 2, 3 and 4. Which species are chosen from within each group is not important. Because group 2 prey are small, composite samples may be required. We do not believe that sampling is required from Sabine NWR, because this area is outside the scope of the BERA.

5.1 Selection of Sampling Locations

Earlier, we argued that group 1 fish and invertebrates (small, lower trophic level species with small home ranges found mostly in shallow areas) are likely to have the highest levels of variability in the concentrations of tissue-associated contaminants. As such, tissue residue levels are likely to reflect the variability in the concentrations of sediment-associated contaminants. We suggest that sampling focus on group I organisms within the sub-areas that have been selected for the sediment quality triad sampling program. This approach would facilitate the estimation of sediment-biota accumulation factors (BSAFs) for each area of concern and sub-area within the study area. BSAFs, so derived, are likely to be useful for developing target clean-up levels for sediments to protect against unacceptable levels of bioaccumulation in the food web.

Unfortunately, expansion of the number of sub-areas that are reflected in the Triad survey to more than 50 would render this approach for tissue sampling unworkable. For this reason, we have collapsed those 50+ sub-areas into 10 primary areas of concern (i.e., excluding Bayou Verdine; Table 4). Based on the results of the power analysis, we estimate that at least 20 samples are required from each area of concern to support evaluations of geographic differences in contaminant concentrations and, hence, risks to ecological receptors. The areas of concern are subsequently divided into one to five sub-areas to characterize potential differences in contaminant concentrations within the various areas of concern. In some cases, the sub-areas include several smaller reaches that were identified for the purposes of sediment quality sampling.

Rules to guide the fish and invertebrate collection during the Phase II sampling program are required. Our plan is to pre-designate sampling locations from the list of randomly-selected sediment sampling sites. At each of these stations, multiple samples of group 1 fish and invertebrates (i.e., 3 to 5 samples of each sub-group) will be collected within a 25 m radius of the designated coordinates. The dimensions of each station will be expanded to 100 m for collecting group 2, 3, and 4 fish samples. The coordinates of alternate sampling stations will also be identified to enable field staff to collect samples at alternate locations if the primary sampling station do not yield sufficient samples.

A summary of the recommended numbers of group 1 invertebrate samples that should be collected from each area of concern and sub-area within the study area is presented in Table 4. As was the case for the group I fish sampling, the group 1 invertebrate sampling is designed to provide data on tissue-associated contaminant concentrations for at least 20 samples from each area of concern. Again, this level of sampling effort is based on the results of the power analyses conducted with the existing tissue residue data. We recognize that invertebrate sampling will be challenging, and thus sampling will occur at fewer locations than with group 1 fish. The dataset should be sufficient, however, to establish the relationship between group 1 fish and invertebrates with regard to levels of tissue associated contaminants. This relationship will facilitate estimation of levels in invertebrate tissues in areas where sampling was not done. Three general groups of invertebrates should be targeted within each area of concern, including bivalves (*Rangia sp.*), small crabs (e.g., hermit crabs, fiddler crabs), and other invertebrates (i.e., polychaetes, other crustaceans, etc.). When possible, relatively equal numbers of samples from each of these three groups should be collected to facilitate evaluations of differences in contaminant concentrations among the three groups of invertebrates. The data on tissue residue levels in invertebrates will be essential for estimating BSAFs for each area of concern within the study area.

For the other groups of fish species (groups 2, 3 and 4), we divide the study area into four areas of concern, including the Upper Calcasieu River, Bayou d'Inde, Middle Calcasieu River, and the Lower Calcasieu River (Table 5). This recommendation reflects the expectation that groups 2, 3, and 4 fish species generally have larger foraging ranges than group 1 fish species and, hence, it will be difficult to sample distinct populations at smaller spatial scales. Sampling in the reference areas within the Lower Calcasieu River system is recommended to support evaluations of baseline levels of risk to focal species within the study area. Each area of concern has been divided into three sub-areas to help focus sampling effort. We recommend that equal numbers of samples be obtained from each sub-area to facilitate subsequent evaluations of differences in contaminant concentrations within areas of concern. Once again, the numbers of samples recommended for collection within each area of concern reflects the results of the power analyses that were conducted on the existing tissue residue data.

Based on the information presented in Tables 4 and 5, we recommend that a total of 692 fish and invertebrate tissue samples be collected to support the BERA. These samples include 276 group 1 fish tissue samples and 164 group 1 invertebrate tissue samples. In addition, 84 tissue samples should be collected to evaluate tissue residues in each of group 2 , 3, and 4 fish. Sampling of group 5 fish is not required to support the BERA. These recommendations do not include the fish that will be needed to support the human health risk assessment or to evaluate fish health.

5.2 Sampling Timing

Some fish and invertebrate sampling methods (i.e., bottom trawling, beach seining) can disrupt *in situ* sediments, accelerate sediment transport, and/or alter the integrity of the benthic community. Therefore, it would be desirable to conduct the sediment quality triad sampling prior to implementing the fish and invertebrate sampling program. However, sampling of fish and invertebrate tissues should be initiated as soon as possible to minimize the effects of fall migration patterns and other climatic-driven events on the distribution of target species. Therefore, the fish and invertebrate sampling program should be initiated on October 25, 2000.

5.3 Sampling Methods

A variety of sampling methods will be employed to capture the fish and invertebrates required for tissue analyses. Some of the methods that could be employed to collect fish and invertebrates include:

Group	Organism Type	Sub-Type	Sampling Methods
1	Invertebrates	crabs	minnow traps, dip nets, mini-trawls
		clams	small oyster or hydraulic (jet) dredges.
		infauna	grab sampling (i.e., Ekman or Van Veen), followed by sieving through 0.5 mm sieves.
1	Fish		minnow traps, small-mesh beach seines, small trap nets
2	Fish		minnow traps, small-mesh beach seines, cast nets, small-mesh gill nets, small trap nets
3	Fish		gill nets, trap nets, beach seines with bag
4	Fish		gill nets, trap nets, beach seines with bag, set lines, angling

5.4 Sample Handling and Preparation

PPG and their sampling team have developed and refined procedures for handling and tracking field-collected fish and invertebrate samples. However, we should ensure that all of the samples collected in an area of concern be held until sampling has been completed within that area. In this way, PPG can provide us with a detailed inventory of the samples (with detailed descriptions of sample types) that have been collected in that area. This will enable us to make decisions regarding the deployment of additional sampling effort and which of the samples that have been collected should be submitted for chemical analysis. All samples should be stored on ice in the field and frozen at -20^{o}C upon receipt at the staging facility.

The analyses of COPCs in fish and invertebrate tissues will be conducted at several analytical laboratories (i.e., metals may be done at one lab, PCBs and OC pesticides at another, and SVOCs at a third). For this reason, there is a need to homogenize fish and invertebrate samples prior to shipment to the analytical laboratories (i.e., to prepare whole sample homogenates and obtain aliquots for shipment to each lab).

Samples should be thawed to facilitate homogenization. All samples should be homogenized whole, except for hermit crabs and bivalves, which should be removed from their shell prior to homogenization. Subsequently, aliquots should be removed for moisture and lipid determinations. In addition, aliquots should be removed to support analyses of each group of chemical analytes. At this stage, sample splits can also be obtained to support determinations of within laboratory (i.e., split samples sent blind to the same lab) and among laboratory (i.e., split samples sent to different labs) analytical precision. If standard reference materials are to used in the QA program, then these samples can be introduced into the sample stream at the staging facility at this time.

5.5 Analyses of Contaminants of Potential Concern

The BERA workshop report (MacDonald et al. 2000) identified the bioaccumulative contaminants of potential concern in the Calcasieu Estuary. These substances include mercury, PAHs, PCBs, PCDDs/PCDFs, chlorinated benzenes, and organochlorine pesticides. In addition to these substances, we recommend adding lead to the list of bioaccumulative COPCs. Levels of metals, PAHs, PCBs, chlorinated benzenes, and organochlorine pesticides should be measured in all tissue samples collected during the monitoring program. We recommend that PCDDs/PCDFs be measured in roughly 20% of the fish and invertebrate tissue samples that are collected, targeting the areas that are known to be contaminated by this group of chemicals (i.e., Lower Bayou d'Inde and, possibly the Coon Island Loop). It may be useful to target PCDDs/PCDFs analyses on Group 4 fish species, as these species are likely to accumulate these substances to elevated levels in their tissues and represent important prey species for birds and mammals that could be adversely affected by dietary exposures to these contaminants.

Some ancillary information is needed to support subsequent interpretation of the tissue residue data. Each sample must include information on the collection location, sampling methods, collection date, and the length and weight of the organism(s) collected. It would also be desirable to obtain information on the sex, age, and reproductive status of fish species. If possible, we recommend that group 1 fish and invertebrates undergo brief examinations for signs of gross pathology (e.g., lesions, fin erosion, tumours, emaciation) and any such signs be recorded. Measurements of moisture content (%) and lipid content (%) are required for each sample.

We anticipate that tissue samples will be sent to the analytical laboratories as homogenates. This will facilitate the introduction of QA samples into the sample stream at the staging facility, including splits, spikes, and standard reference materials. We also anticipate that sample analysis will require several steps, depending on the analyte under consideration. For PCDD/PCDF and PCBs, sample homogenates will be spiked with ^{13}C-labelled PCDD/PCDF/PCB mixtures to facilitate evaluations of % recovery of target analytes. Samples will then be dried with sodium sulphate (or equivalent) and extracted using DCM (or an equivalent organic solvent). Multi-stage sample clean-up will then be required to remove residual lipids and transfer the various chemical analytes into different fractions for quantification by GC-HRMS. Aroclors and OC pesticides will be quantified using GC-ECD. SVOCs will be quantified using GC with a mass selective detector. Mercury concentrations will be determined using the cold-vapour AA method; arsenic, cadmium, and silver will be determined using graphite furnace AA, while other metals will be quantified using flame AA. Methylmercury analyses will be required for invertebrate samples only (>95% of total mercury in fish is methylmercury). All chemical concentrations should be reported on a wet weight basis. **Note: We recommend that target analytical detection limits be established well below published toxicity thresholds (i.e., see SAP) to ensure the relevance of the resulting data in the BERA and minimize the potential for obtaining <DL values.** A high frequency of <DL values makes it difficult to distinguish differences in contaminant concentrations among the various areas of concern. EPA methods, detection limits, and required sample sizes are listed in Table 6 for the COPCs to be analyzed.

5.6 Quality Assurance and Quality Control

Generation of reliable data on the levels of contaminants in fish and invertebrate tissues is an essential component of the overall baseline ecological risk assessment. Some of the considerations for ensuring that reliable data are generated during the Phase II sampling program include:

- Establishing a clear linkage between the BERA and the sampling program design (i.e., through the identification of ecosystem goals, ecosystem objectives, assessment endpoints, and measurement endpoints);
- Conducting a pre-sampling workshop to ensure that field personnel have a complete understanding of where, when, and how sampling is to take place;
- Establishing sampling protocols that must be followed in the field. Such protocols should identify minimum sample volumes, explain equipment preparation and decontamination methods, and describe sample collection, labelling, preservation, and transport procedures. The information that must be documented in the field should be identified in the protocols as well (see SAP);
- Providing technical oversight throughout the sampling program to ensure that the sampling protocols are being carried out as specified;

C Ensuring that field sampling equipment (e.g., Hydrolabs) are adequately maintained and frequently calibrated;

C Implementing a proven system for tracking samples from field collection through laboratory analysis;

C Designing and implementing a blind analytical QA program that includes splits to evaluate analytical precision and spikes to evaluate analytical accuracy. While the analytical laboratories will conduct some sample spiking to evaluate recoveries as part of their internal QA program, inclusion of standard reference materials or samples that have been spiked by a third party will provide an independent basis for evaluating analytical accuracy;

C Identifying target detection limits that are below thresholds for ecological effects and facilitate determination of differences among areas of concern (i.e., detection limits should be low enough to minimize the frequency of <DL values; the treatment of which can significantly influence the results of data analyses – see SAP for list of DLs).

C Developing and implementing a data management system that facilitates data retrieval and minimizes the potential that data will have to be coded more than once (i.e., analytical laboratories should be instructed on the required format for constructing and delivering electronic data files).

6.0 References

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Table 1. Summary of feeding ecology for wildlife focal species.

Species Name	Common Name	Diet	Foraging area	Prey Size	Prey Trophic Level	Notes
Ceryle alcyon	Belted kingfisher	90% fish; 10% invertebrates	2-4 km of shoreline	4-14 cm	2.6-3	usually requires a perch over water; feeds at the water surface
Pandion haliaetus	Osprey	99% fish; 1% other	1.7-10 km radius	11-30 cm	2.5-3.1	other prey includes snakes, small mammals, birds
Ardea herodias	Great blue heron	90% fish; 10% other	0.6-8.4 ha* up to 25 km**	6-41 cm	Aquatic: 2.4-3 Wetland/Terrestrial: 1-3.5	*feeding territory; ** foraging distance from colony
Lutra canadensis	Northern river otter	95% fish; 5% other	10-78 km shoreline	7-5 cm	Aquatic: 2.6-3.2 Wetland: 2-4	prey size is for fish only, river otter are opportunistic and will consume large and small prey as available; other prey items may include turtles, birds (i.e., ducks), invertebrates, etc.
Pelecanus occidentalis	Brown pelican	90% fish; 10% other	typically no more than 30 km out to sea	14-90 cm		primarily feed on 'rough' fish - menhaden, herring, sheepshead, pigfish, mullet, grass minnows, silversides; occasionally feed on prawns
Actitis macularia	Spotted sandpiper	primarily aquatic invertebrates		less than 5 cm		occasionally eat small fish; chicks are fed mostly insects; feeds in the top 4 cm of water
Mustela vison	Mink	opportunistic feeders; diet varies with season and availability	1 to 4 km along shoreline; home range is typically within 300 m of shoreline	7-12 cm		

Species Name	**Common Name**	**Diet**	**Foraging area**	**Prey Size**	**Prey Trophic Level**	**Notes**
Cataptrophorus semipalmatus	Willet	generalist ; both plants and animals; primarily aquatic insects				marine worms, small crabs, small molluscs, fish fry, and small fish; some vegetable matter; forages in the first 10 cm of the water column
Casmerodius albus	Great egret	90% fish; 10% other		up to 100 cm		also feeds on crickets, grasshoppers, snakes, frogs, nice, and some insects
Himantopus mexicanus	Black-necked stilt	90% aquatic invertebrates; 10% fish, reptiles, or amphibians				feeds in the first 20 cm of the water column
Ajaia ajaja	Roseate spoonbill	primarily aquatic invertebrates				also feed on shrimp, molluscs, fish, sheepshead minnows, and insects; typically forage in the first 20 cms of the water column
	Ibis	aquatic invertebrates				crayfish, crabs, fish, fiddler crabs, insects, earthworms, water snakes, and snails
Tursiops truncatus	Bottlenose dolphin	100% fish		12 to 140 cm		herring, pilchard, hake, squid, small bonito, anchovies, sardines; forage in large groups
Procyon lotor	Raccoon	70% terrestrial; 30% wetland/aquatic			Aquatic: 2.2-2.8 Wetland: 2-3.4 Terrestrial:1	in the spring ,diet is more evenly distributed among prey types (e.g., 50% terrestrial/50% wetland/aquatic)

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Species Name	Common Name	Diet	Foraging area	Prey Size	Prey Trophic Level	Notes
Aythya affinis	Lesser Scaup	primarily aquatic vegetation and seeds; lesser quantities of aquatic invertebrates				tend to feed in open water 3 to 8 m deep
Sterna forsteri *Sterna nilotica*	Forster’s Tern Gull-winged Tern	95% fish; 5% invertebrates				Forster’s tern and Gull-winged tern likely found at Calcasieu Estuary; diet is approximation for both species of tern; gull-winged tern is known to consume terrestrial insects ˆ diet ratio may be higher for insects

Table 2. Life history parameters for fish and invertebrate species present in marine, estuarine, and freshwater environments in the coastal Louisiana area.

Species Name	Common Name	Habitat	Length [cm] Max. Length	Diet	Trophic Level	Notes
Family: Ariidae (Sea catfishes)						
Arius felis	Hardhead catfish	Open ocean and estuaries (brackish); Turbid waters over muddy bottom	34.1 60.0	Zoobenthos (TL >= 2.8)	3.1 (se. 2.6 -- 3.6)	
Family: Atherinidae (Silversides)						
Labidesthes sicculus	Brook silverside	Near surface of lakes, ponds, and quiet pools of creeks and small to large rivers, usually in open water	8.8 13.0	Omnivore		Predators include fish
Membras martinica	Rough silverside	Along shore and in bays and inlets	8.5 12.5	Omnivore		
Menidia beryllina	Inland silverside	Marive species that enters rivers; in fresh water, near the surface in quiet water over sand or gravel	10.0 15.0	Omnivore		
Family: Blenniidae (Combtooth blennies)						
Hypsoblennius ionthas	Freckled blenny	Mangroves, pilings, and rocky shores, often in silty water	5.4 7.5	Crustaceans, hydroids, bryozoans, and pelecypods; omnivore	3.2 (se. 2.9 - 3.5)	Marine
Hypleurochilus geminatus	Crested blenny	Demersal	7.0 10.0	Omnivore		Marine
Parablennius marmoreus	Seaweed blenny	Eroded basins and limestone boulders covered by an algal mat	6.1 8.5	Omnivore		Marine

Species Name	Common Name	Habitat	Length [cm] Max. Length	Diet	Trophic Level	Notes
Chasmodes saburrae	Florida blenny	In clumps of oysters and mangrove roots	7.0 10.0	Omnivore		
Hypsoblennius hentz	Feather blenny	Oyster reefs and rocky shores	7.0 10.0	Omnivore		
Family: Centrarchidae (Sunfishes)						
Lepomis punctatus	Spotted sunfish	Heavily vegetated ponds, lakes, pools of creeks, rivers, and swamps; Over mud or sand	12.9	Omnivore		
Lepomis microlophus	Redear sunfish	Ponds, swamps, lakes, and vegetated pools over muddy or sandy bottoms	18.8 30.6	Prefers molluscs; omnivore		
Lepomis humilis	Orange spotted sunfish	Quiet pools or creeks and small to large rivers (often turbid)	10.0 15.0	Omnivore		
Acantharchus pomotis	Mud sunfish	Pools and backwaters of creeks and rivers over mud and detritus	13.5 21	Omnivore		
Lepomis cyanellus	Green sunfish	Quiet pools and backwaters of lakes, ponds, streams, and rivers; in vegetation	19.0 31.0	Omnivore		
Lepomis marginatus	Dollar sunfish	Vegetated pools of rivers and swamps over mud or sand bottom	8.2 12	Omnivore		
Micropterus salmoides	Largemouth bass	Clear vegetated lakes, ponds, and swamps. Also backwaters and pools of creeks and rivers.	52.1 97.0	Fish, crayfish, frogs, crustaceans, insects (TL >= 2.8)	3.8 (se. 3.2 - 4.4)	Predators include herons, bittens, and kingfishers as well as esocids and percids

Species Name	Common Name	Habitat	Length [cm] Max. Length	Diet	Trophic Level	Notes
Elassoma zonatum	Banded pygmy sunfish	Swamps, heavily vegetated sloughs and small sluggish streams	3.6 4.7	Omnivore		
Family: Clupeidae (Herrings, shads, sardines, menhadens)						
Brevoortia patronus	Gulf menhaden	Shore or deep water, feeds at the bottom, wide tolerance to salinity	18.2 25.0	Phytoplankton	2	
Family: Cyprinodontidae (Pupfishes)						
Cyprinodon variegatus	Sheepshead minnow	Hypersaline lagoons and channels over muddy bottoms often turbid	6.4 9.0	Omnivore		Predators include fish
Family: Engraulidae (Anchovies)						
Anchoa mitchilli	Bay anchovy	Shallow tidal areas with muddy bottoms and brackish water	7.0 10.0	Feeds mostly on mysids, copepods, fish, gastropods, and isopods (TL >= 2.8)	3.5 (se. 3.0 - 4.0)	Tolerates a wide range of salinity (from fresh to hypersaline); Predators include jellyfish and fish.
Family : Fundulidae (Topminnows and killifishes)						
Lucania parva	Rainwater killifish	In vegetated quiet water, swims several cms below the surface	3.8 5.0	Omnivore		Predators include other fish
Leptolucania ommata	Pygmy killifish	Surface waters of swamps, vegetated sloughs, and quiet water areas of creeks and small rivers	2.4 2.9	Omnivore		
Fundulus pulvereus	Bayou killifish	In bays, brackish waters, benthopelagic	4.1 5.5	Omnivore		

Species Name	Common Name	Habitat	Length [cm] Max. Length	Diet	Trophic Level	Notes
Fundulus grandis	Gulf killifish	Grassy bays, canals, and nearby fresh water, avoids tidal flats	10.0 18.0	Omnivore		
Adinia xenica	Diamond killifish	Freshwater and hypersaline flats, salt marshes, and mangroves	4.5 6.0	Omnivore		
Family: Gobiidae (Gobies)						
Gobionellus stigmaticus	Marked goby	Very shallow and muddy bottoms of coastal waters, sometimes in the intertidal zone.	5.8 8.0	Omnivore		Euryhaline; Predators include sea birds (*Anous stolidus* and *Sterna fuscata*)
Gobionellus stigmaturus	Spottail goby	Rubbly shallows of open coasts	4.8 6.5	Omnivore		Marine and freshwater
Bollmannia communis	Ragged goby	Demersal	7.0 10.0	Omnivore		
Gobiosoma robustum	Code goby	Sea grass beds and algal mats in very shallow, protected waters.	4.2 5.0	Omnivore		Prefers saline water, but can enter brackish water
Gobiosoma bosc	Naked goby	Estuaries and weedy, protected coastal waters	4.5 6.0	Omnivore		Brackish and marine
Microgobius gulosus	Clown goby	Quiet, muddy waters varying from fresh to fully saline waters, always near water's edge and usually in or near estuaries	5.4 7.5	Omnivore		Predators include fish

Species Name	Common Name	Habitat	Length [cm] Max. Length	Diet	Trophic Level	Notes
Gobionellus boleosoma	Darter goby	Euryhaline species ranging from brackish (almost freshwater) to hypersaline littoral lagoons, in quiet waters of bays and estuaries in grassy and muddy areas	5.4 7.5	Omnivore; zoobenthos (TL >= 2.8)	3.3 (se. 2.9-3.7)	Predators include fish
Microgobius thalassinus	Green goby	Muddy tidepools	0.9 1.0	Omnivore		
Gobionellus shufeldti	American freshwater goby	Low salinity waters of bays and estuaries	5.8 8.0	Omnivore		
Gobioides broussoneti	Violet goby	Muddy bays and estuaries, encountered in freshwater, also offshore on muddy bottoms and near mouths of large rivers	31.7 55.3	Omnivore		
Family: Lepisosteidae (Gars)						
Lepisosteus oculatus	Spotted gar	In quiet, clear pools and backwaters of lowland creeks, small to large rivers, lakes, swamps, and sloughs, can enter brackish waters	59.2 112.0	Fish and crustaceans		
Atractosteus spatula	Alligator gar	Sluggish pools and backwaters of large rivers, swamps, bayous, and lakes, enters brackish and marine waters	143.5 305.0	Fish and crustaceans	4.0 (se. 3.3-4.7)	
Family: Mugilidae (Mullets)						
Mugil cephalus	Flathead mullet	Cosmopolitan in coastal areas, often enters estuaries and rivers, schools over sandy or muddy bottoms and dense vegetation	54	Zooplankton, zoobenthos, and detritus	2.1 (se. 1.9-2.3)	Predators include whales, dolphins, bony fish, sharks and rays, sea birds and shore birds

Species Name	Common Name	Habitat	Length [cm] Max. Length	Diet	Trophic Level	Notes
Mugil curema	White mullet	Sandy coasts and littoral pools, also over muddy bottoms of brackish waters and estuaries, sometimes enters rivers	48	Algae, plankton, detritus		
Family: Paralichthyidae (Large-tooth flounders), Bothidae and Pleuronectidae (Right- and lefteye flounders), Achirdae (Sole), Cynoglossidae (Tonguefish)						
Syacium gunteri	Shoal flounder	Prefers sand or muddy bottoms	12.9 20.0	Omnivore		
Etropus microstomus	Smallmouth flounder	Demersal	8.8 13.0	Omnivore		
Paralichthys lethostigma	Southern flounder	Mainly over mud bottoms in estuaries and coastal waters to about 40 m, tolerates low salinities, frequently in brackish bays and estuaries even in fresh water	45.0 76.0	Fish, crabs, shrimps, and invertebrates		
Paralichthys dentatus	Summer flounder	Prefers hard sandy substrate where they can burrow, exploits estuary habitats including salt marsh creeks and sea grass beds with silty substrate	50.7 94.0	Omnivore		Predators include fish
Citarichthys spilopterus	Bay whiff	Inshore, moves into bays and shallow Gulf during warmer months	Max.=15 cm	Omnivore		
Trinectes maculatus	Hogchoker	Common in bays and shallow Gulf, tolerant of brackish waters	Max.=15 cm	Omnivore		
Symphurus pelicanus	Longtail tonguefish	Midshelf species usually found in deep waters	Max.=8 cm	Omnivore		Small invertebrates
Family: Poeciliidae (Poecilids)						

Species Name	Common Name	Habitat	Length [cm] Max. Length	Diet	Trophic Level	Notes
Poecilia latipinna	Sailfin molly	Ponds, lakes, sloughs, and quiet vegetated backwaters and pools of small rivers, abundant in tidal ditches and brackish canals	10.0 15.0	Feeds mainly on algae; omnivore		
Heterandria formosa	Least killifish	Heavily vegetated standing to slow-moving fresh and brackish water	3.6	Worms, crustaceans, and plant matter		
Crabs						
Callinectes sapidus	Blue crab	Along muddy shores in water of relatively low salinity, in shallow bays or estuaries covered with eel grass		Detritivore, fish		
	Fiddler crabs, hermit crabs, others	Shallow water		Detritivore, carnivore		
Family: Sciaenidae (Drums or Croakers)						
Micropogonias undulatus	Atlantic croaker	Over muddy and sandy bottoms in coastal waters and estuaries	22.4 50.0	Mainly worms, crustaceans, and fish		Predators include fish
Sciaenops ocellatus	Red drum	Over sandy or muddy bottoms in coastal waters and estuaries, sometimes in surf zone	60.6 155	Crustaceans, molluscs, and fish		
Pogonias cromis	Black drum	Over sandy or muddy bottoms in coastal waters, especially in large river runoffs, juveniles enter estuaries	43.8 91.0	Crustaceans and fish	4.5 (se. 3.7-5.3)	
Cynoscion nothus	Gray weakfish or sea trout	Offshore, deeper waters	Max.=30 cm	Crustaceans and fish		Predators include fish

Species Name	Common Name	Habitat	Length [cm] Max. Length	Diet	Trophic Level	Notes
Cynoscion nebulosus	Smalltooth weakfish or sea trout	In deeper areas, often over oyster reefs	58.3 125	Omnivore		Large adults feed on mullets
Cynoscion arenarius	Smalltooth weakfish or sea trout	Common in the bays and shallow Gulf	Max.=41 cm	Piscivore		

Table 3. Listing of prey species belonging to five sampling groups representing various types of prey fish and invertebrates.

GROUP 1 **Small** **Benthopelagic** **Shallow Water** **Localized Distribution** **TL 2 - 2.5**	**GROUP 2** **Small** **Pelagic** **Any Depth** **Broad Distribution** **TL 2 - 2.5**	**GROUP 3** **Large** **Pelagic** **Any Depth** **Broad Distribution** **TL 2 - 2.5**	**GROUP 4** **Medium to Large** **Benthopelagic** **Any Depth** **Broad Distribution** **TL 2.5 - 3.5**	**GROUP 5** **Large** **Pelagic** **Shallow Water** **Localized Distribution** **TL 2.5 - 3.5**
Freckled blenny (*Hypsoblennius ionthas*)	Brook silverside (*Labidesthes sicculus*)	Flathead mullet (*Mugil cephalus*)	Hardhead catfish (*Arius felis*)	Spotted gar (*Lepisosteus oculatus*)
Crested blenny (*Hypleurochilus geminatus*)	Rough silverside (*Membras martinica*)	White mullet (*Mugil curema*)	Shoal flounder (*Syacium gunteri*)	Alligator gar (*Atractosteus spatula*)
Seaweed blenny (*Parablennius marmoreus*)	Inland silverside (*Menidia beryllina*)		Smallmouth flounder (*Etropus microstomus*)	
Florida blenny (*Chasmodes saburrae*)	Gulf menhaden (*Brevoortia patronus*)		Southern flounder (*Paralichthys lethostigma*)	
Feather blenny (*Hypsoblennius hentz*)	Bay anchovy (*Anchoa mitchilli*)		Summer flounder (*Paralichthys dentatus*)	
Sheepshead minnow (*Cyprinodon variegatus*)			Bay whiff (*Citarichthys spilopterus*)	
Marked goby (*Gobionellus stigmaticus*)			Hogchoker (*Trinectes maculatus*)	
Spottail goby (*Gobionellus stigmaturus*)			Longtail tonguefish (*Symphurus pelicanus*)	
Ragged goby (*Bollmannia communis*)			Atlantic croaker (*Micropogonias undulatus*)	
Code goby (*Gobiosoma robustum*)			Red drum (*Sciaenops ocellatus*)	

<u>GROUP 1</u> **Small** **Benthopelagic** **Shallow Water** **Localized Distribution** **TL 2 - 2.5**	**<u>GROUP 2</u>** **Small** **Pelagic** **Any Depth** **Broad Distribution** **TL 2 - 2.5**	**<u>GROUP 3</u>** **Large** **Pelagic** **Any Depth** **Broad Distribution** **TL 2 - 2.5**	**<u>GROUP 4</u>** **Medium to Large** **Benthopelagic** **Any Depth** **Broad Distribution** **TL 2.5 - 3.5**	**<u>GROUP 5</u>** **Large** **Pelagic** **Shallow Water** **Localized Distribution** **TL 2.5 - 3.5**
Naked goby (*Gobiosoma bosc*)			Black drum (*Pogonias cromis*)	
Clown goby (*Microgobius gulosus*)			Gray weakfish or sea trout (*Cynoscion nothus*, *C. arenarius*, *C. nebulosus*)	
Darter goby (*Gobionellus boleosoma*)			Largemouth bass (*Micropterus salmoides*)	
Green goby (*Microgobius thalassinus*)			Blue Crab	
American freshwater goby (*Gobionellus shufeldti*)				
Violet goby (*Gobioides broussoneti*)				
Sailfin molly (*Poecilia latipinna*)				
Least killifish (*Heterandria formosa*)				
Spotted sunfish (*Lepomis punctatus*)				
Redear sunfish (*Lepomis microlophus*)				
Orangespotted sunfish (*Lepomis humilis*)				

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<u>GROUP 1</u> **Small** **Benthopelagic** **Shallow Water** **Localized Distribution** **TL 2 - 2.5**	<u>GROUP 2</u> **Small** **Pelagic** **Any Depth** **Broad Distribution** **TL 2 - 2.5**	<u>GROUP 3</u> **Large** **Pelagic** **Any Depth** **Broad Distribution** **TL 2 - 2.5**	<u>GROUP 4</u> **Medium to Large** **Benthopelagic** **Any Depth** **Broad Distribution** **TL 2.5 - 3.5**	<u>GROUP 5</u> **Large** **Pelagic** **Shallow Water** **Localized Distribution** **TL 2.5 - 3.5**
Mud sunfish (*Acantharchus pomotis*)				
Green sunfish (*Lepomis cyanellus*)				
Dollar sunfish (*Lepomis marginatus*)				
Banded pygmy sunfish (*Elassoma zonatum*)				
Rainwater killifish (*Lucania parva*)				
Plains killifish (*Fundulus zebrinus*)				
Pygmy killifish (*Leptolucania ommata*)				
Bayou killifish (*Fundulus pulvereus*)				
Gulf killifish (*Fundulus grandis*)				
Diamond killifish (*Adinia xenica*)				
Mummichog (*Fundulus heteroclitus*)				

PHASE II SAMPLING DESIGN

<u>GROUP 1</u> Small Benthopelagic Shallow Water Localized Distribution TL 2 - 2.5	<u>GROUP 2</u> Small Pelagic Any Depth Broad Distribution TL 2 - 2.5	<u>GROUP 3</u> Large Pelagic Any Depth Broad Distribution TL 2 - 2.5	<u>GROUP 4</u> Medium to Large Benthopelagic Any Depth Broad Distribution TL 2.5 - 3.5	<u>GROUP 5</u> Large Pelagic Shallow Water Localized Distribution TL 2.5 - 3.5
Clams (*Rangia* spp)				
Small Crabs				

PHASE II SAMPLING DESIGN

Table 3. Approximate expected proportions of diet for each focal species from each prey group.

Focal Species	Group 1	Group 2	Group 3	Group 4	Group 5	Other
Willet	80	0	0	0	0	Plants
Spotted Sandpiper	100	0	0	0	0	
Black-necked Stilt	90	0	0	0	0	Seeds
Lesser Scaup	50	0	0	0	0	Aquatic and terrestrial plants
Great Blue Heron	55	0	0	45	0	
Great Egret	100	0	0	0	0	
Roseate Spoonbill	100?	?	0	0	0	Group 2?
Ibis	100	0	0	0	0	
Belted Kingfisher	100	0	0	0	0	
Osprey	5	15	15	65	0	
Brown Pelican	0	50	50	0	0	
Terns	25	50	25	0	0	
Bottlenose Dolphin	0	80	20	0	0	
Mink	60	0	0	10	0	Terrestrial organisms
Northern River Otter	30	0	0	40	0	Birds, turtles, etc
Raccoon	30	0	0	0	0	Terrestrial organisms

Table 4. Phase II Biota Sampling in the Calcasieu Estuary:
Recommended Sampling Locations and Density: Group 1 Invertebrates and Fish.

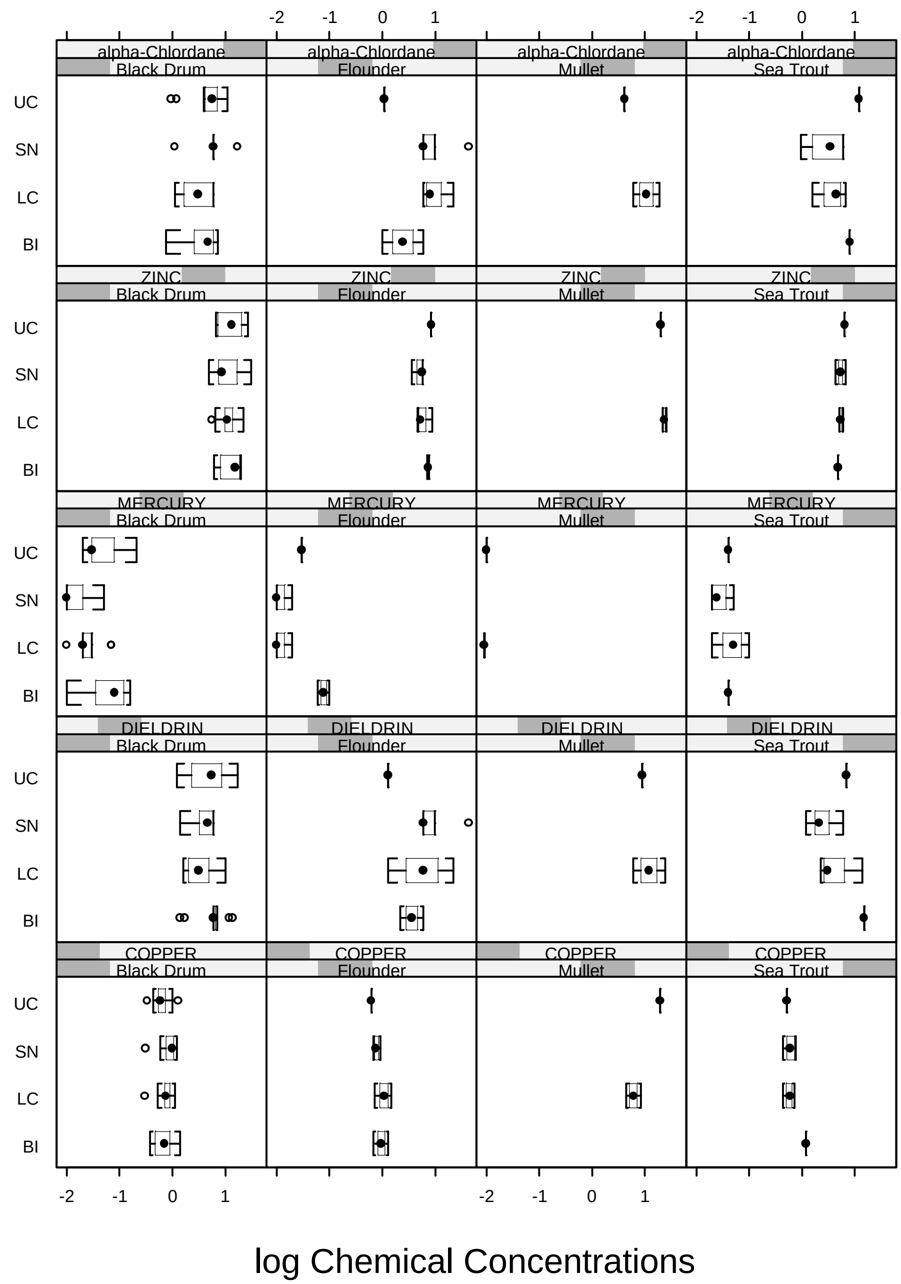
Area of Concern	Sub-Area	Number of Sampling Stations	Number of Samples Required	
			Fish	Invertebrates
1. Upper Calcasieu River	Lake Charles	5	20	0
2. Clooney Island Loop	Loop and Barge Slips	5	20	20
3. Coon Island Loop	Coon Island NW	3	12	12
	Coon Island NE	3	12	12
4. Upper Bayou d'Inde	UBI 1 and 2	2	8	0
	UBI 3, 4, and 5	3	12	12
5. Middle Bayou d'Inde	MBI 1 and 2	2	8	6
	MBI 3 and 4	2	8	6
	MBI 5, 6, and 7	3	12	9
6. Lower Bayou d'Inde	PPG Canal and associated wetlands	3	12	6
	LBI 1, 2, and 12	3	12	6
	LBI 3, 6, 10, and 13	4	16	0
	LBI 4, 5, 7, and 9	4	16	8
	LBI 8, 11, and 14	3	12	6
7. Prien Lake	Prien Lake and old river channel	5	20	0
8. Middle Calcasieu River	Citgo Surge Pond	5	20	20
9. Moss Lake	Bayou Olsen	3	12	12
	Moss Lake	2	8	8

10. Lower Calcasieu Reference Areas	Choupique Bayou and wetlands	3	12	9
	Bayou Connine Bois	3	12	6
	Grand Bayou and wetlands	3	12	6
All Areas			**276**	**164**

Table 5. Phase II Biota Sampling in the Calcasieu Estuary:
Recommended Sampling Locations and Density: Group 2, 3, 4, and 5 Fish.

		Number of Samples Needed			
Area of Concern	**Sub-Area**	**GRP-2**	**GRP-3**	**GRP-4**	**GRP-5**
1. Upper Calcasieu River	Lake Charles	7	7	7	0
	Clooney Island Loop & Barge Slips	7	7	7	0
	Coon Island Loop	7	7	7	0
2. Bayou d'Inde	Lower Bayou d'Inde	7	7	7	0
	Middle Bayou d'Inde	7	7	7	0
	Lower Bayou d'Inde	7	7	7	0
3. Middle Calcasieu River	Prien Lake and Old River Channel	7	7	7	0
	Moss Lake and Bayou Olsen	7	7	7	0
	Citgo Surge Pond	7	7	7	0
4. Lower Calcasieu Reference Areas	Choupique Bayou and wetlands	7	7	7	0
	Bayou Connine Bois	7	7	7	0
	Grand Bayou and wetlands	7	7	7	0
All Areas	**All Sub-Areas**	**84**	**84**	**84**	**0**

Figure 7. Box plot of log chemical concentrations (mg/kg for zinc, copper and mercury; µg/kg for chlordane and dieldrin) for five chemicals in four fish species from the Phase I sampling program. UC=Upper Calcasieu, SN=Lower Calcasieu, SN=Sabine National Wildlife Refuge, and BI=Bayou d'Inde.



PHASE II SAMPLING DESIGN

Figure 8. Box plot of the coefficient of variation for log chemical concentrations (mg/kg for zinc, copper and mercury; µg/kg for chlordane and dieldrin) for five chemicals in four fish species from the Phase I sampling program. UC=Upper Calcasieu, SN=Lower Calcasieu, SN=Sabine National Wildlife Refuge, and BI=Bayou d'Inde.

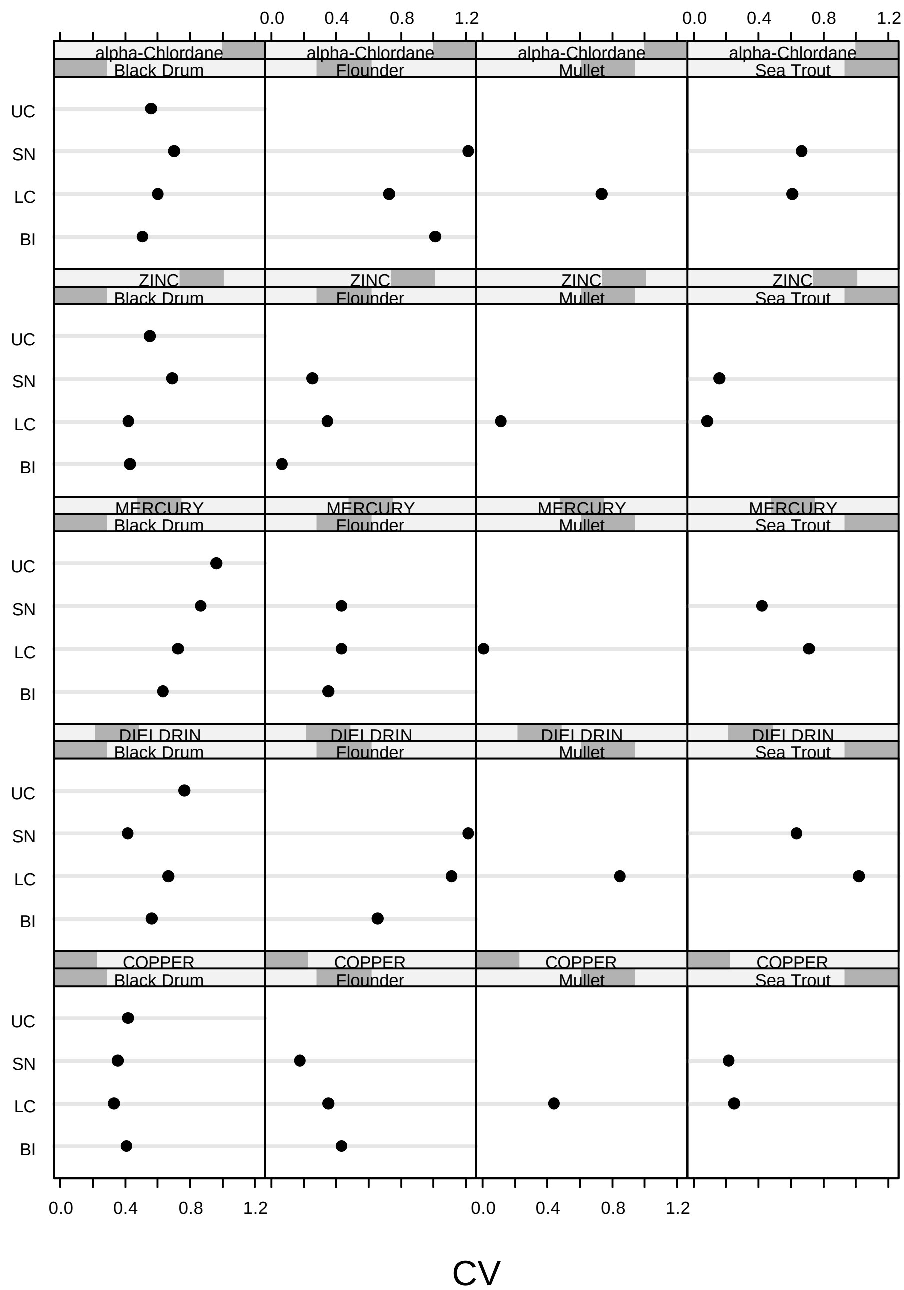


***Figure 9*.** Power curves for detecting differences varying from d = log 0.1 to log 1 based upon Phase I results for tissue levels of mercury in black drum.

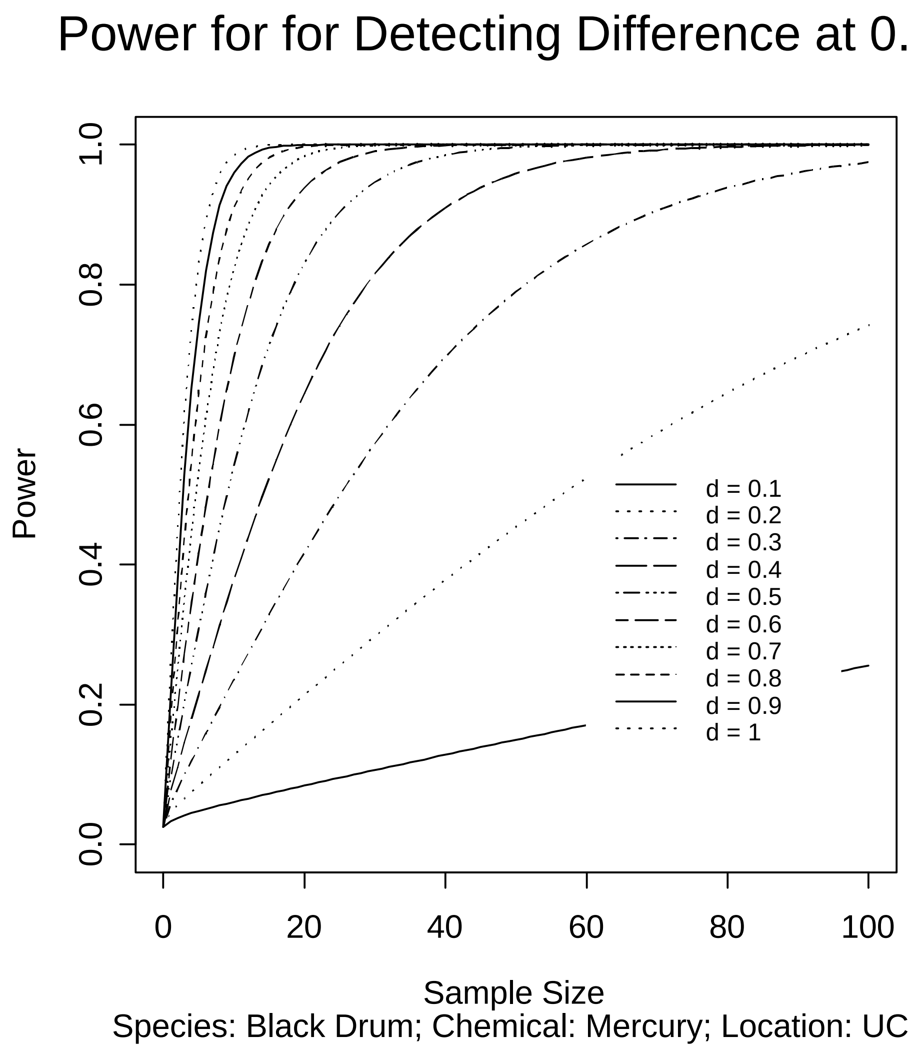


Figure 10. Power curves for detecting differences varying from d = log 0.1 to log 1 based upon Phase I results for tissue levels of copper in black drum.

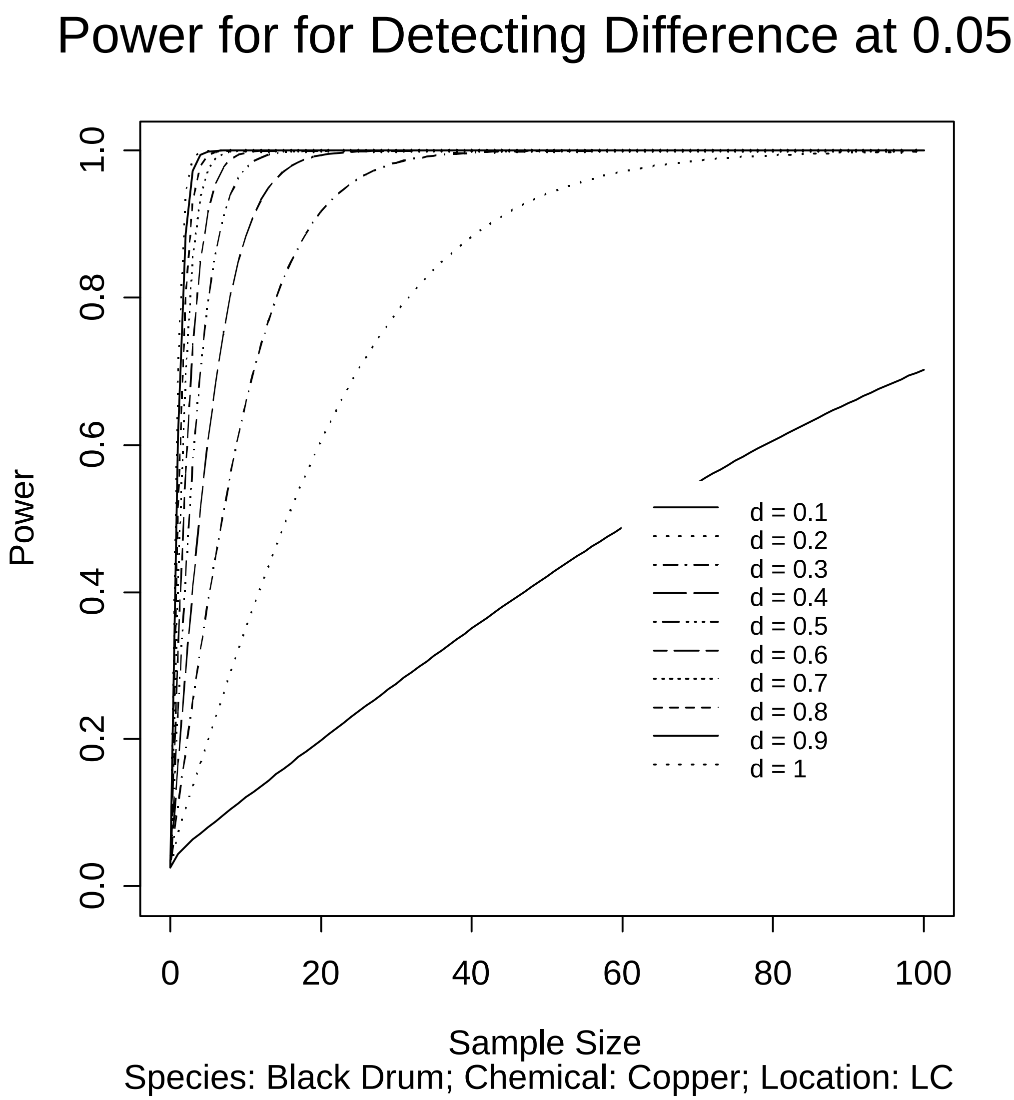


Figure 11. Power curves for detecting differences varying from d = log 0.1 to log 1 based upon Phase I results for tissue levels of dieldrin in flounder.